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Prevalence of Genes Encoding Pyrogenic Toxin Superantigens and Exfoliative Toxins among Strains of *Staphylococcus aureus* Isolated from Blood and Nasal Specimens

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A total of 429 different *Staphylococcus aureus* isolates encompassing 219 blood isolates and 210 isolates taken from anterior nares were systematically searched by two multiplex PCR-DNA enzyme immunoassays (PCR-DEIA) for exfoliative toxin (ET) genes *eta* and *etb*, as well as for the classical members of the pyrogenic toxin superantigen (PTSAg) gene family comprising the staphylococcal enterotoxin (SE) genes *sea-see* and the toxic shock syndrome toxin 1 gene *tst*. In addition, a third PCR-DEIA was established to investigate the possession of four recently described SE genes, viz. *seg-sej*. The most frequent PTSAg/ET genes amplified were *seg* and *sei*, which were found strictly in combination in 55.0% of the *S. aureus* isolates tested. Other frequently detected toxin genes were *tst* (20.3%), *sea* (15.9%), and *sec* (11.2%). Only five isolates harbored ET genes. Regarding the origin of the *S. aureus* isolates, a significant difference ($P = 0.037$) was found for the possession of the *sed*/*sej* gene combination (10.5% of blood isolates versus 3.3% of nasal strains). Overall, about half of *S. aureus* isolates tested harbored genes of the classical members of the PTSAg family and ETs (50.8%), whereas 73.0% of *S. aureus* isolates were toxin gene positive if the recently described SE genes were included. This notable higher prevalence indicates that the possession of PTSAg genes in particular seems to be a habitual feature of *S. aureus*. Moreover, mainly due to the fixed combinations of *seg* plus *sei*, as well as *sed* plus *sej*, the possession of multiple PTSAg genes (62.9%) is more frequent than assumed so far.

Staphylococcus aureus, which has its ecologic niche in the anterior nares, has been shown to cause a variety of infectious diseases ranging from superficial skin infections to severe systemic infections including toxin-mediated diseases (23). By virtue of exfoliative toxins (ETs) and pyrogenic toxin superantigens (PTSAgs) comprising toxic shock syndrome toxin 1 (TSST-1) and the staphylococcal enterotoxins (SEs), *S. aureus* causes the staphylococcal scalded-skin syndrome (SSSS), the staphylococcal toxic shock syndrome (TSS), and staphylococcal food poisoning (SFP) (3, 7). Besides their specific toxic properties, the members of the PTSAg family share several structural features and biological characteristics, such as pyrogenicity, superantigenicity, and the ability to enhance the susceptibility to endotoxin shock (20, 36).

In addition to the classical five major antigenic types of SEs (SEA, SEB, SEC, SED, and SEE) (11), four additional SEs (SEG, SEH, SEI, SEJ) have been reported, and their corresponding genes have been described (30, 34, 42). For SEC and SEG, several subtypes have been characterized (1, 24). Most recently, the "alphabet" of the SE family was expanded by the detection of further genes (*sek*, *sel*, *sem*, and *seo*) encoding enterotoxin homologues (18, 21, 31).

In previous studies, the prevalence of classical-toxin-producing *S. aureus* strains has been investigated by conventional methods based on immunological procedures measuring the toxins in the culture supernatants of suspected strains (22, 27, 37). As a consequence of the availability of commercial tests, these studies were restricted to five classical enterotoxins: SEA to SED and TSST-1. Furthermore, the immunological methods used thus far are known to be limited in sensitivity and specificity (6, 16, 32). To date, no valid data are available concerning the prevalence of the more recently described enterotoxins in *S. aureus* isolates derived from clinical specimens.

Molecular methods offer alternative ways to detect *S. aureus* isolates with the genetic information to produce toxins irrespective of their expression. Previously, we described the rapid and specific detection of toxigenic *S. aureus* isolates by the use of two multiplex PCR-DNA enzyme immunoassays (PCR-DEIA) for amplification and hybridization of the classical ET genes (*eta* and *etb*), the SE genes (*sea*, *seb*, sec_1 to sec_3, *sed*, and *see*), and the TSST-1 gene (*tst*) (4). For the study reported here, this approach was extended by developing a further multiplex PCR-DEIA targeting SEG-SEJ genes (*seg*, *seh*, *sei*, and *sej*).

The goals of the present study were (i) to extend the two multiplex PCR-DEIAs by a third one targeting the newly described SE genes, (ii) to investigate the prevalence of PTSAgs and ETs in a well-defined *S. aureus* strain collection obtained during the course of a German multicenter study, and (iii) to analyze possible differences in toxin gene equipment between colonizing isolates and isolates involved in severe systemic infection.

MATERIALS AND METHODS

Bacterial strains. A total of 429 *S. aureus* isolates were collected and identified during the course of a German multicenter study that included general and intensive care units at 32 university and community hospitals (40). All 219 blood

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TABLE 1. Base sequences, gene locations, and predicted sizes of PCR products for the oligonucleotide primers to amplify specifically fragments of the SE genes *seg* to *sej*

Gene[a]	Primer	Oligonucleotide sequence (5′-3′)	Location within gene (nucleotide range)	Size of PCR product (bp)
seg[b]	SEG-1W	AAT GCY CAA CCY GAT CCT A	223–241	116
	SEG-4	CTT CCT TCA ACA GGT GGA GAC	318–338	
seh	SEH-1	TTA GAA ATC AAG GTG ATA GTG GC	407–429	235
	SEH-2	TTT TGA ATA CCA TCT ACC CAA AC	619–641	
sei	SEI-1	GCC ACT TTA TCA GGA CAA TAC TT	469–491	330
	SEI-2	AAA ACT TAC AGG CAG TCC ATC TC	776–798	
sej	SEJ-1	CTC CCT GAC GTT AAC ACT ACT AAT AA	940–965	641
	SEJ-2	TTG TCT GGA TAT TGA CCT ATA ACA TT	1580–1605	

[a] Sequences and locations were derived from the published nucleotide sequences for *seg* (30), *seh* (34), *sei* (30), and *sej* (42).
[b] The primer sequences used here cover also the nucleotide sequence of variant SEG (1).

isolates obtained from patients with *S. aureus* bacteremia during the multicenter study were included here. The most frequently clinically presumed causes of bacteremia were catheter-related infections (46%); osteomyelitis, skin and soft tissue infections such as cutaneous abscesses, and cutaneous ulcerations (27%); and lower-respiratory-tract infections (11%). In addition to the blood isolates, a total of 210 *S. aureus* nasal isolates prospectively collected over a 6-year period during routine surveillance were also tested for the exotoxin genes. None of the patients who were nasally colonized and who were selected for the present study subsequently developed *S. aureus* bacteremia within that study period (40). Twenty (9.1%, exhibiting nine genotypes) of the patients with *S. aureus* bacteremia and six (2.9%, exhibiting five genotypes) of the *S. aureus* nasal carriers harbored methicillin-resistant *S. aureus* strains.

DNA isolation procedures. Staphylococcal cells were collected from 0.5 ml of an overnight brain heart infusion broth culture. Cells were pelleted by centrifugation at 5,000 × *g* for 20 min, resuspended in 185 μl of TE buffer (20 mM Tris chloride, 2 mM EDTA [ph 8.0]) with 15 μl of recombinant lysostaphin (15 mg/ml; Sigma, St. Louis, Mo.), and incubated at 37°C for 30 min. DNA was subsequently extracted with a QIAamp tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Nucleic acid samples were eluted with distilled water and adjusted to a final concentration of 1 μg/ml according to A_{260} values.

Multiplex PCR-DEIA procedure. Both the multiplex PCR-DEIA to detect simultaneously the classical SE genes (*sea*, *seb*, sec_1 to sec_3, *sed*, and *see*) and the multiplex PCR-DEIA to detect the ET genes (*eta* and *etb*) and the TSST-1 gene (*tst*) were performed as previously described (4). The newly designed oligonucleotide primers to detect the recently described SE genes *seg*, *seh*, *sei*, and *sej* by a third multiplex PCR procedure are listed in Table 1. The assay conditions for these genes corresponded with that of the published multiplex PCR assays. For carryover prevention, the uracil DNA glycosylase system (Boehringer Mannheim, Mannheim, Germany) was used for all multiplex PCR assays. The amplification was performed in an iCycler (Bio-Rad, Munich, Germany). For hybridization of the amplified DNA generated by the multiplex PCR assays, a generic DNA enzyme immunoassay (GEN-ETI-K DEIA; Sorin, Saluggia, Italy) was used according to the manufacturer's recommendations and as described elsewhere (4). Four additional probes were developed to control the specificity of the multiplex PCR by using reference strains harboring *seg* to *sej*. (Table 2). Positive controls for the PCR-DEIAs included DNA from toxin-producing *S. aureus* reference strains (Table 3). In addition to standard PCR controls for contamination events, *S. aureus* Cowan 1 and *S. epidermidis* DSM 20044 served as negative controls.

Fingerprinting by ERIC-PCR. Genotyping of *sed*/*sej*-positive isolates by using ERIC2 primer for the enterobacterial repetitive intergenic consensus (ERIC) sequence was performed as described by van Belkum et al. (39) with modifications concerning the thermal cycling conditions and the use of 0.5 U of AmpliTaq DNA polymerase, Stoffel fragment (Perkin-Elmer, Branchburg, N.J.). The thermal cycling conditions were 35 cycles of denaturation at 94°C for 1 min (5 min for the first cycle), annealing at 35°C for 1 min, and polymerization at 72°C for 2 min. Drastically prolonged ramp times (5 min) were used (12). Amplified products (10 μl) were resolved by 2% agarose gel electrophoresis at 150 V for 1.5 h. The gel was stained with ethidium bromide and then exposed to UV light (254 nm) to visualize the amplified products.

Statistical analysis. Differences between groups were assessed by using the chi-square test. If more than one exotoxin gene in various combinations was found, multivariate analysis by logistic regression was used to establish their importance (10). Epi-Info 2000 (Centers for Disease Control and Prevention, Atlanta, Ga.) was used to perform calculations. An odds ratio higher than 1 within a confidence interval of 95% were taken into account for associations. *P* values of <0.05 were considered statistically significant.

RESULTS

Evaluation of the detection system. The previously published PCR results (4) and the newly established multiplex PCRs with primer pairs specifically for (i) *sea* to *see*; (ii) *tst*, *eta*, and *etb*; and (iii) *seg* to *sej* genes successfully amplified fragments from the DNA of *S. aureus* reference strains (Fig. 1 and Table 3). PCR amplifications were confirmed by the respective hybridization reactions in the DEIA system for all reference strains used (Table 3). The sensitivity of the multiplex PCR-DEIAs reached the expected range, as previously described (4). None of the primer pairs and the respective hybridization

TABLE 2. Base sequences and gene locations for the *seg*-*sej*-specific 5′-biotinylated oligonucleotide probes

Gene[a]	Probe[b]	Oligonucleotide sequence (5′-3′)	Location within gene (nucleotide range)
seg[c]	SEG-8B	GAT TCA TTA CAT TAC CCA TAG TTC CC	555–580
seh	SEH-3B	ACT GCT GAT TTA GCT CAG AAG TTT A	454–478
sei	SEI-3B	ACA CTG GTA AAG GCA AAG AAT ATG	656–679
sej	SEJ-3B	GGG TAT CTC TGA AAA GAT AAT GAC A	1087–1111

[a] Sequences and locations were derived from the published nucleotide sequences for *seg* (30), *seh* (34), *sei* (30), and *sej* (42).
[b] 5′ biotinylated.
[c] The probe sequence used here covers also the nucleotide sequence of variant SEG (1).

TABLE 3. Results of testing reference strains for SE genotypes *seg* to *sej* derived from agarose gel analysis of multiplex PCR and colorimetric microtiter plate DEIA

Reference strain	Toxin genotype determined by previous study	Genotype as determined by:								Reference or source
		Agarose gel analysis[a]				Colorimetric assay[b] (OD ratio at 450/630 nm)				
		seg	*seh*	*sei*	*sej*	*seg*	*seh*	*sei*	*sej*	
ATCC 13565	*sea*[c]	−	−	−	+	0.035	0.118	0.035	1.617	ATCC
ATCC 14458	*seb*	−	−	−	−	0.035	0.044	0.042	0.070	ATCC
ATCC 19095	*sec*	+	+	+	−	1.023	2.127	>2.500	0.071	ATCC
ATCC 23235	*sed*	+	−	+	+	2.399	0.022	2.384	2.361	ATCC
ATCC 27664	*see*	−	−	−	−	0.056	0.090	0.120	0.076	ATCC
MJB 1320	*sei*[d,e]	−	−	+	−	0.033	0.048	>2.500	0.053	30
FRI 445	*seg sei*[d,f]	+	−	+	−	2.228	0.052	>2.500	0.052	28, 30
FRI 569	*seh*[d]	−	+	−	−	0.047	2.131	0.105	0.054	38
FRI 572	*seg sei*[d]	+	−	+	−	1.976	0.045	>2.500	0.062	28, 30, 38
FRI 1472	*sed seg sei sej*[d]	+	−	+	+	2.346	0.039	>2.500	2.067	42
KN813	*tst*	+	−	+	−	0.139	0.045	0.067	0.058	4
BM 10458	*eta*	−	−	−	−	0.080	0.079	0.083	0.077	4
BM 10143	*etb*	−	−	−	−	0.096	0.074	0.129	0.065	4
Cowan 1[g]	Nontoxigenic	+	−	+	−	0.070	0.046	0.083	0.085	4
ATCC 20044[g]	Nontoxigenic	−	−	−	−	0.036	0.041	0.033	0.079	4

[a] −, negative; +, positive (as judged by eye).
[b] Reactions were judged as positive if the signal reached or exceeded the cutoff value of 0.150 absorbance units above the mean value of determinations of toxin-negative reference strains.
[c] In our study, this was also tested as positive for SED by using reversed passive latex agglutination and EIA and was confirmed by a positive PCR result testing *sed.*
[d] Strains were addionally tested by PCR and DEIA (data not shown) as completely negative for the classical exotoxin genes *sea* to *see*, *tst*, *eta*, and *etb*, except for strain FRI 1472, which also exhibited *sej*-positive PCR amplification and DEIA hybridization for the *sed* gene (42), as well as for the *seg* and *sei* genes.
[e] This strain is only *sei* positive due to the possession of the pMJB471 plasmid encoding *sei* gene (30).
[f] In contrast to the observation of Munson et al. (30) regarding SEH production of FRI 445, PCR targeting of the *seh* gene resulted in a negative reaction in our study, as also reported by Monday and Bohach (28).
[g] Negative control strains; nontoxigenic *S. epidermidis* ATCC 20044 and *S. aureus* Cowan I were considered nontoxigenic for classical PTSAgs and ETs.

probes reacted with the negative control strain *Staphylococcus epidermidis* ATCC 20044. *S. aureus* Cowan 1, shown to be nontoxigenic regarding the classical PTSAg and ET genes (4), was found to be PCR positive for *seg-sei* gene combination but was below the detection point for DEIA hybridization for both genes. The same findings regarding the *seg-sei* combination were observed for *S. aureus* KN 813, which is known to be *tst* positive (Table 3). It is worth noting that amplification products with primers specific for *sed* and *sej* were found for *S. aureus* ATCC 13565 in addition to the detection of the *sea* gene. To date, this strain has been described only as SEA positive (American Type Culture Collection [ATCC] catalog, 19th edition) and has not been recognized as possessing the *sed-sej* gene combination. This finding was confirmed by a respective hybridization procedure. Production of SED by this strain was verified by staphylococcal enterotoxin test-reversed passive latex agglutination and Ridascreen-EIA as previously described (4). The possession of the newly described SE genes was also detected for ATCC strains 19095 and 23235, hitherto only known to be *sec* and *sed* positive, respectively (ATCC catalog) (Table 3).

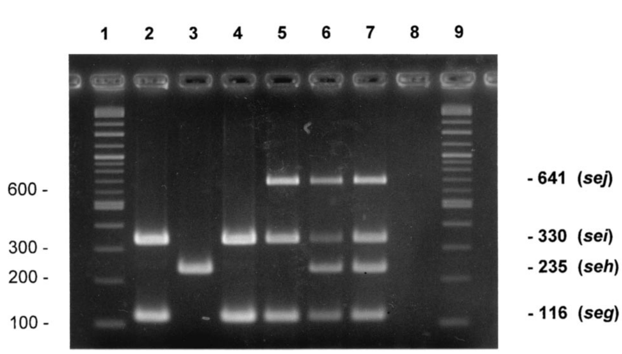

FIG. 1. Agarose gel electrophoreses patterns showing PCR-amplified products in multiplex PCR for the SE genes *seg*, *seh*, *sei*, and *sej*. Lanes: 1 and 9, DNA molecular weight marker (1 kb/100-bp DNA ladder); 2, FRI 572 (*seg* and *sei* positive); 3, FRI 569 (*seh* positive); 4, FRI 445 (*seg* and *sei* positive); 5, FRI 1472 (*seg*, *sei*, and *sej* positive); 6, multiplex PCR with all enterotoxin genes simultaneously (*seg* to *sej*); 7, artificial arrangement of the amplification fragments of *seg* to *sej*; 8, *S. epidermidis* ATCC 20044 (negative control). Sizes are indicated in base pairs on the left and right of the figure.

Detection of PTSAg and ET genes. Data on the prevalence of PTSAg and ET genes testing 429 clinical *S. aureus* isolates by multiplex-PCRs are shown in Table 4. Overall, 313 (73.0%) isolates were positive for at least one of the exotoxin genes tested.

The most commonly found genes were the more recently described enterotoxin genes *seg* and *sei* (n = 236 [55.0%]), which were strictly found in association with one another. Regarding the classical enterotoxin genes, the *tst* gene (n = 87 [20.3%]) was the most frequently detected gene, followed by *sea* (n = 68 [15.9%]) and *sec* (n = 48 [11.2%]). Fragments of the *sed* gene were amplified in a total of 30 isolates (7.0%), always in combination with the newly described enterotoxin gene *sej*. Isolates harboring other PTSAg genes were infrequently recovered (Table 4).

The possession of more than a single PTSAg and/or ET gene was observed in the majority of both nasal and blood isolates (62.9%); this was mainly due to the fixed combinations of *seg* plus *sei* (n = 236 [55.0%]) and *sed* plus *sej* (n = 30 [7.0%]) (Table 5). Overall, a multitude of PTSAg gene combinations

TABLE 4. Results of testing 429 clinical *S. aureus* isolates for staphylococcal PTSAg and ET genes by multiplex PCR

Result of PCR testing	No. and percentages of isolates						P^a
	Blood (n = 219)		Nasal swabs (n = 210)		Total (n = 429)		
	n	%	n	%	n	%	
sea positive	38	17.4	30	14.3	68	15.9	0.756
seb positive	13	5.9	16	7.6	29	6.8	0.214
sec positive	19	8.7	29	13.8	48	11.2	0.911
sed positive	23	10.5	7	3.3	30	7.0	**0.037**
see positive	1	0.5	1	0.5	2	0.5	0.902
seg positive	116	53.0	120	57.1	236	55.0	0.062
seh positive	10	4.6	13	6.2	23	5.4	0.290
sei positive	116	53.0	120	57.1	236	55.0	0.062
sej positive	23	10.5	7	3.3	30	7.0	**0.037**
tst positive	40	18.3	47	22.4	87	20.3	0.498
eta positive	1	0.5	4	1.9	5	1.2	ND
etb positive	0	0.0	2	1.0	2	0.5	ND

[a] The P values were assessed by multivariate analysis by using logistic regression. ND, not done. Boldface values indicate significant differences between blood and nasal isolates.

were observed, including isolates with the combination of two (n = 115), three (n = 103), four (n = 46), or five (n = 6) different genes. Apart from the fixed gene combinations, the most frequent combination detected was *seg-sei* plus *tst* occurring in 8.6% of all isolates. Altogether, 80 (18.6%) isolates harbored *tst* in various combinations, including *sea-seg-sei* (5.8%), *seh-seg-sei* (1.4%), and several other gene combinations. In contrast, seven (1.6%) isolates were observed with *tst* only. Further frequent nonfixed gene combinations were found for *sea*-positive isolates (11.9%) and *sec*-positive isolates (10.0%). Similar to *tst*, the sole presence of *sea* (4.0%) or *sec* (1.2%) is much less common than their presence in combination with other toxin genes. The second frequent gene combination was *seg-sei* plus *sec* (7.7%), followed by *seg-sei* plus *sea* and *tst* (5.8%). The *seg-sei* gene combination was detected in all isolates simultaneously bearing four (n = 46) or five (n = 6) different PTSAg genes. Overall, only 42 (9.8%) of all isolates tested harbored a single PTSAg gene, representing 13.5% of all PTSAg gene-positive isolates.

Among the 429 isolates examined, only five (1.2%) isolates were positive for one or both ET genes tested (Tables 4 and 5). Of these, one *eta*-positive blood isolate was found that also carried the *seg-sei* gene combination. The other four ET gene-positive isolates were derived from nasal swabs. Two of these isolates harbored both *eta* and *etb* genes (Table 5).

Comparing blood and nasal isolates. The association between the detection of PTSAg and ET genes and the origin of isolation was studied by multivariate analysis (Table 4). No

TABLE 5. Single and multiple possession of staphylococcal PTSAg and ET genes

SE genotype	*S. aureus* PTSAg/ET gene-positive isolates					
	Blood (n = 219)		Nasal swabs (n = 210)		Total (n = 429)	
	n	%	n	%	n	%
Single possession						
sea	9	4.1	8	3.8	17	4.0
seb	7	3.2	3	1.4	10	2.3
sec	3	1.4	2	1.0	5	1.2
seh	1	0.5	2	1.0	3	0.7
tst	3	1.4	4	1.9	7	1.6
eta	0	0.0	1	0.5	1	0.2
Total[a]	23	10.5	20	9.5	43	10.0
Multiple possession[b]						
sea (in combination)	29	13.2	22	10.5	51	11.9
seb (in combination)	6	2.7	13	6.2	19	4.4
sec (in combination)	16	7.3	27	12.9	43	10.0
sed-sej (in combination)	23	10.5	7	3.3	30	7.0
see (in combination)	1	0.5	1	0.5	2	0.5
seg-sei (in combination)	116	53.0	120	57.1	236	55.0
seh (in combination)	9	4.1	11	5.2	20	4.7
tst (in combination)	37	16.9	43	20.5	80	18.6
eta (in combination)	1	0.5	3	1.4	4	0.9
etb (in combination)	0	0.0	2	1.0	2	0.5
Total	140	63.9	130	61.9	270	62.9
Total positive[c]	163	74.4	150	71.4	313	73.0
Subset of classical PTSAg or ET genes[d]	111	50.7	107	51.0	218	50.8
Total negative[e]	56	25.6	60	28.6	116	27.0
Subset of classical PTSAg or ET genes[f]	108	49.3	103	49.0	211	49.2

[a] Total of isolates with exclusive possession of only one of the PTSAg or ET genes tested. No clinical isolate was observed with sole possession of *sed*, *see*, *seg*, *sei*, *sej*, or *etb*.
[b] That is, possession of a gene or a fixed gene combination (*sed-sej* and *seg-sei*, respectively) in combination with further exotoxin genes tested.
[c] Positive PCR result for one or more of all staphylococcal PTSAg and ET genes tested.
[d] Positive PCR result for at least one of the classical PTSAg genes (*sea*, *seb*, *sec*, *sed*, *see*, and *tst*) and/or the ET genes (*eta* and *etb*).
[e] Negative PCR result for all staphylococcal PTSAg and ET genes tested.
[f] Negative PCR result for classical PTSAg and ET genes tested.

significant difference ($P = 0.782$) was found with regard to the overall possession of all genes tested between blood isolates ($n = 163$ [74.4% PCR positive)] and those isolates derived from nasal swabs ($n = 150$ [71.4% PCR positive]). This was also true for the group of isolates that were PCR negative in assays of only the classical PTSAg and ET genes ($P = 0.975$). However, an analysis of the subgroups showed that the *sed-sej* gene combination was significantly more present in blood culture isolates (odds ratio = 2.65; 95% confidence interval = 1.1 to 6.6; $P = 0.037$). The likelihood ratio chi-square test showed a value of 22.85 with 7 degrees of freedom ($P = 0.0027$). Genotyping of *sed-sej*-positive isolates by ERIC-PCR (data not shown) revealed that the 23 *sed-sej*-positive blood isolates belonged to 18 genotypes, since the seven nasal isolates accounted for four genotypes. Three of the *sed-sej*-possessing blood isolates were identified as methicillin-resistant *S. aureus* representing different genotypes.

DISCUSSION

Epidemiological studies of well-defined clinical *S. aureus* populations by molecular methods for the investigation of both classical and newly described PTSAg and ET genes are generally lacking. Therefore, the presence of these genes was studied in 429 well-characterized *S. aureus* isolates (40).

Regarding classical staphylococcal members of the PTSAg family, the overall percentage was ca. 10% higher compared to data given in the literature (22, 27, 37). However, most previous studies were based on the detection of toxin production. The fact that toxin gene-positive strains tested negative in immunological assays may be caused either by real nonproduction of the respective toxins or by a weak expression below the detection limit (2, 5). It is well documented that as little as smallest amounts of PTSAgs may induce T-cell stimulation triggering systemic illness such as PTSAg-induced shock and autoimmunity (14, 26, 29). Furthermore, it is known that toxin production can be influenced by many factors that do not influence molecular methods (19). Thus, strains without detectable production of PTSAgs in vitro should not be debarred from epidemiological studies.

Whereas nearly all strains of *S. aureus* produce enzymes and hemolysins that contribute to their pathogenicity, it has been generally accepted that only some strains produce ETs and PTSAgs (8, 11). However, considering both the newly published SE genes (*seg*, *seh*, *sei*, and *sej*) and the classical toxins, the overall rate of toxin gene-positive isolates in the study presented here reached 73.0%. More than half of the isolates (55.0%) harbored the combination of *seg* and *sei* either alone (20.7%) or in further combinations with other toxin genes tested. Recently, Monday and Bohach (28) showed in a limited number of isolates ($n = 15$) carrying the *seg* determinant that this gene is linked to the *sei* gene, which is located 2,002 bp upstream of *seg*. For all 236 *seg-sei*-positive isolates, this fixed combination was confirmed in the context of our study. In contrast, McLauchlin et al. (25) reported on 25 strains, isolated from suspected SFP incidents, that harbored *seg* alone. Possible explanations for these contradictionary observations may be the special food-borne origin of these isolates or the existence of still-unknown variants of *sei* gene as already known for *sec* and *seg* (1, 24). Sequence variations might explain why some isolates tested positive for *seg* and *sei* by PCR but remained negative by using the respective DEIA gene probes.

A further coexisting gene combination was recently reported for the SED-encoding plasmid which encodes a further SE designated SEJ. The SEJ gene is transcribed in a direction opposite to *sed* and is separated by an 895-bp intergenic region (42). The suggestion of Zhang et al. (42) that the *sej* determinant may be present on all *sed*-encoding plasmids originally tested on six strains could be confirmed in our study with 30 *sed*-positive isolates, which always tested as *sej* positive.

The role of the recently described SEs as causative agents in toxin-mediated staphylococcal diseases or in enhancing the severity of *S. aureus* infections still remains unclear. Nevertheless, the isolation of *S. aureus* strains implicated in outbreaks of SFP without producing any of the classical SEs but possessing genes encoding SEG to SEJ has been described (25). In *S. aureus* strains isolated from patients with TSS or SSSS but known to be negative for the classical PTSAgs and ETs, Jarraud et al. (17) detected the *seg-sei* gene combination. Thus, these authors suggested that both SEs might be capable of causing TSS or SSSS. In view of our results, this has to be further investigated since more than 50% of all isolates tested possess these genes.

In addition to the fixed combinations *seg-sei* and *sed-sej*, nonfixed PTSAg/ET combinations were found to be more common than has been reported in the literature (22, 41). Overall, the possession of more than one of the genes tested (including fixed combinations) was more frequent (62.9%) compared to the presence of only a single gene (10.0%). In contrast to a previous report (35), *seh* was found in association with a number of other PTSAg genes such as *sea*, *seb*, *sec*, *seg-sei*, and *tst*.

Regarding ET encoding genes, the low rates of positive isolates (*eta* [1.2%] and *etb* [0.5%]) found here are in accordance with the results of other investigations even though epidemiological data on *S. aureus* strains producing epidermolytic toxins are scarce (9, 13, 15).

Upon comparing *S. aureus* isolates derived from blood versus carriage isolates, we found no significant differences with regard to the overall possession of all PTSAg- and ET-encoding genes tested. However, when we analyzed the prevalence of single genes in both study populations, the fixed gene combination *sed-sej* was significantly more common in blood isolates ($P = 0.037$). By genotyping the *sed-sej* carrying isolates, these isolates were found to belong predominantly to different clones. This finding is in agreement with a previous observation reporting on five of six SED-producing isolates originated from blood cultures (22). Most recently, Peacock et al. showed (33) that *sej* contributed independently to the virulence of *S. aureus*.

Our study is the first systematic and comprehensive search for the classical, as well as for the recently described, PTSAg and ET genes in a large number of well-characterized clinical *S. aureus* isolates. Genes of the PTSAg family were shown to be considerable more common in *S. aureus* than was assumed thus far. Thus, toxinogenic or superantigenic abilities seem to be a habitual feature of this pathogen. Except for the *sed-sej* gene combination, which was more present in blood isolates, no significant differences were found upon comparison of blood and nasal isolates. The potential contribution of *sed-sej* to the overall pathogenicity potential of *S. aureus* should be investigated further.

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